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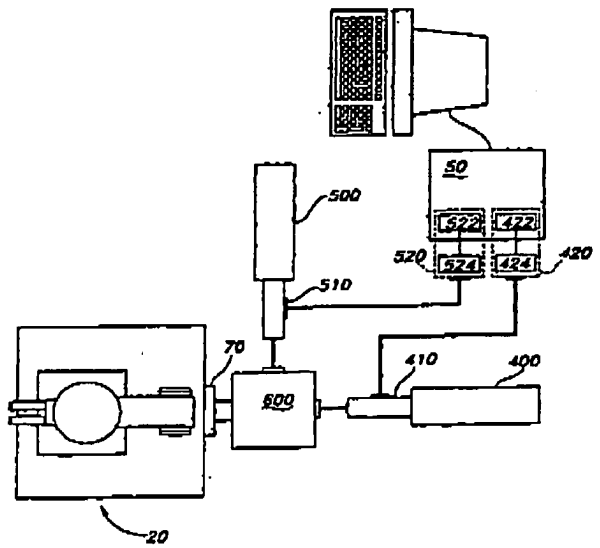

(57) Abstract: A direct-view optical microscope system is provided which uses high-energy light from a phenomenon known as non-resonant Raman scattering to illuminate a living biological specimen. One embodiment of the system combines two discrete light sources to form a combined incident light source for the microscope. The system includes a method and apparatus for modulating the intensity of the scattered light when two light waves are combined to produce the incident light. By varying the frequency of the two source light waves, the intensity of the combined Raman-scattered light can be modulated to achieve finer resolution.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

HIGH-RESOLUTION OPTICAL MICROSCOPE

Technical Field

The present invention relates generally to the field of direct-view optical microscopes and, more particularly, to a method and apparatus for using high-energy light from a phenomenon known as non-resonant Raman scattering to illuminate a living biological specimen.

Background of the Invention

Since their invention in the late 1500s, light microscopes have enhanced our knowledge of basic biology, biomedical research, medical diagnostics, and materials science. Although the science of microscopy has advanced to include a variety of techniques to enhance resolution, the fine-resolution observation of living biological specimens has remained elusive.

Continuing advances in microbiology require a closer and closer study of biochemical events that occur on a cellular and intracellular level. The challenge in microscopy today is not only the enhancement of finer and finer resolution, but also the development of techniques for observing biochemical events in real time, as they happen, without destroying the biological specimen in the process.

Resolution is the ability of a microscope to distinguish between two objects that are very close together. A microscope with a resolution of 1,000 Å (1,000 Angstroms; equal to 100 nanometers or 100×10^{-9} meters), for example, can make objects as close together as 100 nanometers independently visible. Objects and features smaller than 100 nanometers cannot be resolved (*i.e.*, distinguished) by this microscope. Below is a list of the resolution or practical resolving power of several types of microscopes currently available:

2,000 Å	Visible Light Microscope
1,000 Å	Ultraviolet Microscope
150 to 300 Å	Scanning Electron Microscope
2.0 to 4.0 Å	Transmission Electron Microscope

Although electron microscopes offer very fine resolution, the specimen must be prepared by high-vacuum dehydration and is subjected to intense heat by the electron beam, making observation of living specimens impossible. The dehydration process also alters the specimen, leaving artifacts and cell damage that were not present in nature. Also, In order to view the steps in a biological process, dozens of specimens must be viewed at various stages in order to capture each desired step in the process. The selected specimens must then be prepared. Specimen preparation can take up to two hours each.

The high cost of an electron microscope represents another barrier to its use in the life sciences. Electron microscopes are large and often require an entire room. The operation and adjustment of an electron microscope requires highly-skilled technicians, introducing yet another cost of maintaining and staffing an electron microscopy facility.

The ultraviolet microscope offers finer resolution and better magnification than an ordinary light microscope, but it has serious disadvantages for the study of living specimens. Ultraviolet light damages or kills many kinds of living biological specimens, making observation impossible.

When ultraviolet light strikes a specimen, it excites fluorescence within the molecules of the specimen so that the specimen itself emits a fluorescent light. If the specimen does not produce fluorescence naturally, it must be stained with a fluorescent dye. Many fluorescent dyes bind strongly to elements such as enzymes within living cells, changing their qualities and significantly altering the cellular biochemistry. Other dyes produce too much fluorescence or absorb too much of the ultraviolet light to be useful.

Like electron microscopes, the operation of an ultraviolet microscope requires a great deal of skill. Because ultraviolet light damages the human eye, the image can only be observed by ultraviolet video cameras or specially-equipped still cameras. Also, the quartz optics required for ultraviolet microscopes are much more expensive than the glass components used in visible light microscopes.

The electron and ultraviolet microscopes available today do no offer a technique for observing living, unaltered biological specimens in real time.

The Nature of Light

Light is sometimes referred to as a type of electromagnetic radiation because a light wave consists of energy in the form of both electric and magnetic fields. In addition to the light we can see, the electromagnetic spectrum includes radio waves, microwaves, and infrared light at frequencies lower than visible light. At the upper end of the spectrum, ultraviolet radiation, x-rays, and gamma rays travel at frequencies faster than visible light.

Wavelength is the distance between any two corresponding points on successive light waves. Wavelength is measured in units of distance, usually billionths of a meter. The human eye can see wavelengths between 400 and 700 billionths of a meter. Frequency is the number of waves that pass a point in space during any time interval, usually one second. Frequency is measured in units of waves per second, or Hertz (Hz). The frequency of visible light is referred to as color. For example, light traveling at 430 trillion Hz is seen as the color red.

The wavelength of light is related to the frequency by this simple equation (Equation One),

$$f = \frac{c}{L},$$

where c is the speed of light in a vacuum (299,792,458 meters per second), f is the frequency in Hz, and L is the wavelength in meters.

Microscope Resolution

The resolution or resolving power of a light microscope can be calculated using Abbe's Formula,

$$D = \frac{L}{2(NA)},$$

where D is the resolving power of a microscope in meters, L is the wavelength in meters of the light source, and NA is the numerical aperture of the microscope. The numerical aperture, generally, indicates the angle at which light strikes the specimen being viewed.

Light Scattering

When a light wave passes through a specimen, most of the light continues in its original direction, but a small fraction of the light is scattered in other directions. The light used to illuminate the

specimen is called the incident light. The scattering of incident light through various specimens was studied by Lord John William Strutt, the third Baron Rayleigh (Lord Rayleigh) in the late 1800s and later by Albert Einstein and others.

Lord Rayleigh observed that a fraction of the scattered light emerges at the same wavelength as the incident light. Because of his observation, light that is scattered at the same wavelength as the incident light is a phenomenon called Rayleigh scattering (also called resonant scattering or elastic light scattering).

In 1922, Arthur H. Compton observed that some of the scattered light has a different wavelength from the incident light. Compton discovered that, when light passes through a specimen, some of the light scatters off the electrons of the specimen molecules, producing scattered light in the X-ray region of the spectrum.

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Raman Scattering

In 1928, Professor Chandrasekhara V. Raman and Professor K.S. Krishnan discovered that the scattered light observed by Compton was caused by vibrations within the molecules of the specimen. Because of his discovery, light that is scattered due to vibrations within the molecules of a specimen is a phenomenon called Raman scattering (also called non-resonant or inelastic light scattering). In 1930, Raman received the Nobel Prize in Physics for his discovery.

When a specimen is bombarded with incident light, energy is exchanged between the light and the molecules of the specimen. The molecules vibrate, producing the phenomenon known as Raman scattering. The molecular vibrations cause the specimen itself to emit scattered light, some of which scatters at a higher frequency ($f+\Delta f$) than the incident light frequency (f), and some of which scatters at a lower frequency ($f-\Delta f$). The Δf represents the change in frequency (sometimes called the frequency shift) produced by Raman scattering.

In summary, when incident light strikes a specimen, the scattered light includes Rayleigh-scattered light at the same frequency (f) as the incident light, higher frequency ($f+\Delta f$) Raman-scattered light, and lower-frequency ($f-\Delta f$) Raman-scattered light.

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Intensity Depends on the Specimen

Because Raman-scattered light is produced by molecular vibrations within the specimen, the intensity of the Raman-scattered light varies depending upon the type of specimen being viewed. For example, a specimen of blood cells may produce high-intensity Raman-scattered light, while a specimen of skin cells may produce very low-intensity Raman-scattered light.

Raman scattering is used in a variety of spectroscopy systems to study the interaction between a sample and certain types of incident light. The fact that Raman scattering varies depending on the specimen, however, has limited its direct use in the field of microscopy. Although the phenomenon of light scattering is present whenever light strikes a specimen, none of the microscopy systems available today are configured to fully harness the resolving power of Raman scattering.

Thus, there is a need in the art for a microscopy system that takes full advantage of the Raman scattering phenomenon as a source of illuminating a specimen.

There is a related need for a system for relaying and capturing the images produced by such a microscope. There is yet another related need in the art for producing and adapting the types of incident light best suited for provoking Raman scattering in a biological specimen.

There is also a need in the art for a direct-view, optical microscope with a higher resolution and magnification than is currently available.

There is further a need for an optical microscope that provides a real-time image of living biological materials, including cells and intracellular structures. There is a related need for a microscope that permits observation by the human eye and recording by readily-available photomicrographic and video equipment.

There is also a need to provide a system and method for viewing living biological specimens in their natural state, without interference from the artifacts of specimen preparation, without destroying or altering sensitive biochemical characteristics, and without killing the specimen.

There is still further a need for a high-resolution microscope that is less expensive, easy to operate, requires little or no

specimen preparation, and is relatively portable and small enough for use in the field.

Summary of the Invention

The above and other needs are met by the present invention which, stated generally, provides a direct-view optical microscope system that uses high-energy light from a phenomenon known as non-resonant Raman scattering to illuminate a living biological specimen.

In one aspect of the present invention, a microscope system for observing a specimen includes an optical microscope, a light source, a darkfield condenser to focus the light on the specimen, and a compound relay lens connected to the eyepiece of the microscope. The light source is ultraviolet in one embodiment. The system may also include an adapter positioned between the light source and the microscope to align the light. The system may also include a camera and a computer.

The compound relay lens of the present invention includes two relay lenses connected together to provide higher magnification than a single relay lens alone.

In another aspect, the invention provides of method of provoking enough light scattering to illuminate a specimen in an optical microscope system. The method includes illuminating a lamp that emits ultraviolet light, focusing the ultraviolet light upon the specimen using a darkfield condenser, and then magnifying the image of said specimen using said compound relay lens. The method may further include adapting the ultraviolet light for use in the microscope by positioning an adapter between the lamp and the darkfield condenser.

The method may also include the double oil immersion technique, which includes the steps of placing a drop of oil on the underside center of the slide on which the specimen rests, positioning the slide on the center of the darkfield condenser, placing a drop of oil on the top center of the cover glass, and then raising the darkfield condenser until the oil on the top of said cover glass contacts the objective lens.

In another aspect of the present invention, a microscope system is provided for illuminating and observing a specimen with

scattered light from a combined light source. This system includes an optical microscope, a first light wave traveling at a first frequency, a second light wave traveling at a second frequency, an optical combiner to combine the two light waves into one, and a darkfield condenser. The combined light wave includes an additive light wave traveling at an additive frequency and a subtractive light wave traveling at a subtractive frequency. The darkfield condenser focuses the combined light upon the specimen such that the additive and subtractive light waves provoke scattered light.

In one embodiment of the two-light system, the first light wave is produced by a first light filtering system that includes a first light source emitting an unrefined light wave, a first filter, and a first filter controller. The filter controller sends a first control signal to the first filter based upon the desired frequency. The first filter then refines the unrefined light wave into a first light wave traveling at a first frequency. The second light wave is produced by a similar second light filtering system.

The two-light system may also include a compound relay lens, a camera, and a computer. In one embodiment, the two-light system includes an optical combiner. According to the present invention, the optical combiner includes a chamber, a casing enclosing said chamber and including several input ports and an output port, and a prism assembly configured to combine two incoming light waves into a single, combined light wave and project it through the output port.

In another aspect of the two-light system of the present invention, a system for producing the first and second light waves includes a dual-channel filter and a dual-frequency filter controller. The filter controller is configured to send a primary and a secondary control signal to the filter. The dual-channel filter broadcasts the first light wave on a first channel in response to the primary control signal and, in an alternating fashion, broadcasts the second light wave on a second channel in response to the secondary control signal.

In one embodiment, each control signal produces a corresponding acoustic wave inside the dual-channel filter. The first acoustic wave interacting with the unrefined light wave produces the first light wave, and the second acoustic wave interacting with the unrefined light wave produces the second light wave.

In another embodiment, the dual-frequency filter controller includes a primary radio frequency synthesizer, a secondary radio frequency synthesizer, and a driver connecting both synthesizers to the dual-channel filter. Each radio frequency synthesizer is configured to synthesize and send a control signal via the driver to the dual-channel filter.

In another aspect of the present invention, an optical combiner for combining two light waves to produce a single combined light wave includes a chamber, a casing enclosing said chamber and including several input ports and an output port, and a prism assembly configured to combine two incoming light waves into a single, combined light wave and project it through the output port.

In one embodiment, the optical combiner also includes a beam expander connected to each input port designated for light waves emitted by a laser. The beam expander focuses and collimates each incoming laser beam before it reaches the prism.

In an alternative embodiment, the optical combiner is capable of combining a laser light wave and an ultraviolet light wave. The optical combiner is also capable of receiving a single light wave entering through any one of the input ports, and projecting the single light wave through the output port.

In another aspect of the present invention, a method of modulating the combinatory phenomenon to illuminate and view a specimen in an optical microscope system with a combined light includes the steps of filtering a first unrefined light wave to produce a first light wave traveling at a first frequency, filtering a second unrefined light wave to produce a second light wave traveling at a second frequency, combining the light waves into a combined light wave, condensing the combined light, and focusing the combined light upon the specimen. The combined light wave includes an additive light wave traveling at an additive frequency and a subtractive light wave traveling at a subtractive frequency.

The method may also include placing a lower oil drop on the underside center of the slide, positioning the slide on the center of the darkfield condenser, placing an upper oil drop on the top center of the cover glass, and raising the darkfield condenser until the upper oil drop contacts the objective lens of the microscope.

Thus, it is an object of the present invention to provide a microscopy system that takes full advantage of the Raman light scattering phenomenon as a source of illuminating a specimen. It is a related object of the present invention to effectively relay the images captured by such a microscope system for maximum magnification.

It is also an object of the present invention to produce the types of incident light best suited for provoking light scattering in a biological specimen.

It is a further object of the present invention to provide an optical microscope that provides a real-time image of living biological materials, including cells and intracellular structures, that permits direct observation by the human eye, and that facilitates recording by readily-available photomicrographic and video equipment.

It is another object of the present invention to provide a system and method for viewing living biological specimens in their natural state, without interference from the artifacts of specimen preparation, without destroying or altering sensitive biochemical characteristics, and without killing the specimen.

It is also an object of the present invention to provide a fine-resolution, high-magnification microscope that is less expensive, easier to operate, more portable, and less labor-intensive in terms of specimen preparation than ultraviolet, electron, or other types of microscopes.

These and other objects are accomplished by the apparatus, method, and system disclosed and will become apparent from the following detailed description of one preferred embodiment in conjunction with the accompanying drawings.

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Brief Description of the Drawing

Fig. 1 is a diagrammatic side view of a microscope system according to an embodiment of the present invention.

Fig. 2 is a diagrammatic side view of a compound relay lens according to an embodiment of the present invention.

Fig. 3 is a detailed view of the incident light as it passes through a darkfield condenser, strikes a specimen, and enters an optical microscope, according to an embodiment of the present invention.

Fig. 4 is an overhead schematic view of a microscope system according to an embodiment of the present invention.

Fig. 5 is an overhead schematic view of the light waves passing through an optical combiner and entering a microscope, according to an embodiment of the present invention.

Fig. 6 is a graphical representation of the electromagnetic spectrum.

Fig. 7 is an overhead schematic view of an embodiment of the present invention that includes a dual-frequency acousto-optic filter controller.

Fig. 8 is a detailed view of the combined light wave as it passes through a darkfield condenser, strikes a specimen, and enters an optical microscope, according to an embodiment of the present invention.

Fig. 9 is a photomicrograph of a diatom illuminated by an embodiment of the microscope system of the present invention, compared to diatom images in Figs. 9a and 9b obtained by other microscopes.

Figs. 10a, 10b, and 10c are photomicrographs of a micrometer, an optical gage, and a carbon grating illuminated by an embodiment of the microscope system of the present invention.

Fig. 11 is a perspective view of one embodiment of the microscope system according to the present invention.

Figs. 12 and 13 are photomicrographs of blood cells illuminated by an embodiment of the microscope system of the present invention.

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Detailed Description

Reference is now made to the drawing figures, in which like numerals refer to like elements throughout the several views. Fig. 1 shows one embodiment of an optical microscope system 10 according to the present invention. (Fig. 11 is a perspective view of one embodiment of the system 10). The system 10 shown in Fig. 1 includes a first light source 400, an adapter 70, a darkfield condenser 60, a direct-view optical microscope 20, a compound relay lens 30, a camera 40, and a computer 50. The first light source 400 emits a first light 430 which is called the incident light 300 once it enters the microscope 20.

A direct-view optical microscope 20 generally includes a base, a field diaphragm 22, a field condenser such as the darkfield

condenser **60** shown, a stage **24** upon which a specimen may be placed, at least one objective lens **26**, and at least one eyepiece for viewing or otherwise receiving the image captured by the objective lens **26**. The term eyepiece includes a broad range of viewing devices beyond those which involve or are intended for the human eye. Light enters the objective lens **26** and travels into the trinocular head **27**, which comprises an ocular eyepiece pair **28** for viewing with the eye and an upwardly-directed projection eyepiece **29**.

The Compound Relay Lens

In one aspect of the inventive system **10** of the present invention, a compound relay lens **30** is added to the microscope **20** to magnify the image before it enters the camera **40**, as shown in **Fig. 1**. A computer **50** receives the image.

A closer, schematic view of the compound relay lens **30** is shown in **Fig. 2**. The compound relay lens **30** generally includes a first relay lens **32** and a second relay lens **34**. In one embodiment, the first relay lens **32** is a commercially-available objective lens having a cylindrical body and a C-type mount. The second relay lens **34** is a commercially-available relay lens. In a preferred embodiment, the first relay lens **32** has a numerical aperture of 0.65 and a magnification power of 40X, such as the Olympus model A40X objective lens. The second relay lens **34** has a magnification power of 10X, such as the Edmund model L37-820 relay lens. It should be understood that the compound relay lens **30** of the present invention contemplates the use of other types of lenses in combination with one another to produce an increased magnification of the image as it exits any of the eyepieces of the microscope **20**. The combination of these lenses **32, 34** provides greater magnification than either lens would provide alone.

The Light Illuminating the Specimen

In the system **10** as shown in **Fig. 1**, a first light source **400** is used. In one embodiment, the first light source **400** is an ultraviolet light source **100**, which emits a first light **430** having a frequency in the ultraviolet range of the electromagnetic spectrum (see **Fig. 6**). As depicted in **Fig. 1**, the first light **430** is called the incident light **300** once it enters the microscope **20**.

When an ultraviolet light source **100** is used, the system **10** includes an adapter **70** which acts as an interface between the light source **100** and the visible-light optical microscope **20**. The adapter **70** may include an enclosure such as a cylinder, with polished interior walls, and is configured to align the ultraviolet light source **100** with the entrance port of the microscope **20**.

Fig. 3 provides a closer view of the stage **24** of the microscope **20**, where the specimen **200** sits upon a slide **25**. The ultraviolet first light **430** (now referred to as the incident light **300**) enters the darkfield condenser **60** of the microscope **20**. Each darkfield condenser **60** has a numerical aperture value **NA**, which indicates the angle at which light exits the condenser **60**. A Naessens Darkfield Condenser having a numerical aperture **NA** of 1.41 produces excellent results, although other darkfield condensers may be used.

The darkfield condenser **60** generally includes an annular stop **62** and a condenser lens **64**. In general, a darkfield condenser **60** directs the incident light **300** toward the specimen **200** at an angle that prevents most of the incident light **300** from entering the objective lens **26** of the microscope **20**. The annular stop **62** is shaped like a disc and centrally mounted. Understanding the flow of light actually occurs in three dimensions, a hollow cylinder of light passes around the edges of the annular stop **62** and strikes the condenser lens **64**, which bends the light toward the specimen **200** at an angle indicated by the numerical aperture **NA**. The incident light **300** exiting the condenser lens **64** is shaped like a hollow cone. By centering and adjusting the vertical position of the condenser **60**, the cone of light can be positioned and focused such that its vertex strikes the specimen **200**.

Scattered light is produced when the darkfield condenser **60** focuses the incident light **300** directly on the specimen **200**. When the incident light **300** strikes the specimen **200**, most of the light passes through and continues in its original direction, but a small fraction of the light is scattered in other directions. It is primarily the scattered light that enters the objective lens **26** of the microscope **20**.

The scattered light, as shown in **Fig. 3**, includes a Rayleigh component **310**, a high-frequency Raman component **320**, and a low-frequency Raman component **330**. The Rayleigh-scattered light **310** is emitted at the same frequency (**f**) as the incident light **300**. The high-frequency Raman-scattered light **320** is emitted at a higher

frequency ($f+\Delta f$). The lower-frequency Raman-scattered light **330** is emitted at a lower frequency ($f-\Delta f$).

The microscope system **10** shown in **Fig. 1** is designed to take advantage of the high-energy light produced by Raman scattering **320** and use it to illuminate the specimen **200**. It should be understood that types of light other than ultraviolet may be used in the system **10** of the present invention to excite Raman scattering to illuminate a specimen **200**.

The Method

The method of using the microscope system **10** of the present invention produces sufficient scattered light **310, 320, 330** to illuminate a living biological specimen. An ultraviolet light enters the microscope **20** through an adapter **70** and is focused directly upon the specimen **200** by a darkfield condenser **60**. The resulting image is magnified by a compound relay lens **30** and transmitted to a camera **40** and a computer **50**, where the image may be further refined.

One method of using the system **10** includes the general steps of illuminating an ultraviolet light source **100** such as a mercury lamp, adapting the ultraviolet light for use in a visible-light microscope **20**, and focusing the incident light **300** using a darkfield condenser **60** to provoke Raman-type light scattering to illuminate a living biological specimen **200**. The method further includes magnifying the image using a compound relay lens **30** positioned between the microscope **20** and the camera **40**.

In a preferred embodiment, the method of focusing the incident light **300** with the darkfield condenser **60** further includes a technique known as double oil immersion to enhance performance. A low-viscosity, low-fluorescence immersion oil is preferable. Preferably, a very thin cover glass **125** is positioned on top of the specimen **200**, such that the specimen is sandwiched between the slide **25** and the cover glass **125**.

The double oil immersion technique includes placing a drop of oil on the underside of the slide **25** and a drop of oil on the center of the cover glass **125**. When the slide **25** is placed on the microscope stage **24**, the oil on the underside will make immediate optical contact with the condenser **60**. When the stage **24** is carefully raised until the oil on the top of cover glass **125** makes contact with the

objective lens **26**, all optical contacts will occur simultaneously and the specimen **200** will be illuminated.

In this position, as shown in the inset portion of **Fig. 3**, only the width of the lower oil drop **65** separates the condenser **60** from the slide **25** as it rests upon the stage **24** of the microscope **20**. On the upper side, only the width of the upper oil drop **165** separates the cover glass **125** over the specimen **200** from the objective lens **26**.

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The Energy of Scattered Light

The higher frequency ($f+\Delta f$) Raman-scattered light waves **320** possess more energy than the incident light **300**. Referring briefly to **Fig. 6**, the electromagnetic spectrum, it can be appreciated that higher-frequency, shorter-wavelength light waves possess higher energy. Because higher-energy light waves generally improve the resolution **D** of a microscope system **10**, it is desirable to provoke a high amount of high-energy Raman-scattered light **320**.

The intensity of Raman-scattered light **320**, however, is about one-thousandth the intensity of Rayleigh-scattered light **310**. Accordingly, it takes a very powerful (high energy and high frequency) light source to produce enough Raman-scattered light **320** to illuminate a specimen. Unfortunately, using a powerful light source also increases the amount of Rayleigh-scattered light **310**, which can overpower and interfere with the Raman-scattered light **320**.

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Combining Two Light Sources

In another embodiment of the system **10** of the present invention, a method and apparatus is provided for maximizing Raman-type scattering while minimizing the interfering effects of Rayleigh-type scattering. In this embodiment, two light sources are combined, as shown in **Fig. 4**, to produce a combinatory phenomenon. The frequency of each light source can be adjusted to maximize the intensity of the Raman-scattered light **320** produced by the particular specimen **200** being viewed.

For example, although a specimen **200** of skin cells may produce a limited amount of Raman-scattered light **320** when illuminated by a single ultraviolet light source **100**, using two adjustable light sources **400**, **500** can increase the amount and intensity

of Raman-scattered light **320** produced and, thus, increase the resolution **D** of the microscope system **10**.

Referring to **Fig. 4**, a schematic view of this embodiment of the system **10** is depicted. The microscope system **10** includes a first light source **400**, a second light source **500**, an optical combiner **600**, an adapter **70**, and a direct-view optical microscope **20**.

The first light source **400** is filtered by a first acousto-optic tunable filter **410** which is controlled by a first filter controller **420**, which may be housed in a computer **50**. Similarly, the second light source **500** is filtered by a second acousto-optic tunable filter **510** which is controlled by a second filter controller **520**, which may be housed in a computer **50**.

In one configuration, both the first and second light sources **400, 500** are lasers. The light emitted by a laser is well-suited to being filtered to a single frequency, and also well-suited for transmission using fiber optic cable. The laser may be an Argon-ion or Krypton-ion laser such as are available from Omnichrome Corporation, although other types of laser sources may be used.

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The Acousto-Optic Tunable Filter (AOTF)

Referring to the schematic light wave diagram in **Fig. 5**, the first and second tunable filters **410, 510** are used to filter the light from the light sources **400, 500** and produce monochromatic (single-color, single-frequency) light waves **430, 530**. The first light **430** travels at a first frequency f_1 and has a corresponding first wavelength L_1. Similarly, the second light **530** travels at a second frequency f_2 and has a corresponding second wavelength L_2. The corresponding frequencies f_1, f_2 and wavelengths L_1, L_2 may be readily calculated using Equation One (frequency equals the speed of light divided by the wavelength).

A first acousto-optic tunable filter **410** (AOTF **410**) is used in the system **10** of the present invention to filter a light source **400**, typically a laser beam, so that it emits a single-frequency light **430**. The acousto-optic tunable filters **410, 510** may use a Tellurium Dioxide crystal and a transducer, and may be configured specifically to filter light from a laser, such as the fiber-pigtailed laser acousto-optic tunable filter, model TEAF 3-0.45-65-1FP, manufactured by Brimrose Corporation of America. It should be understood, however, that any

device capable of receiving a light wave and filtering it into a single-frequency light may be used as the AOTF **410, 510.**

The first AOTF **410** uses an acoustic wave to shift or change the frequency of the light waves in the laser beam from the first light source **400.** The second AOTF **510** operates in a similar manner upon the second light source **500.** The acoustic wave acts like a filter, interacting with the optical light waves and separating a single frequency of light from all the others. By varying the frequency of the acoustic wave, the frequency of the separated light can be varied. The frequency of the acoustic wave produced in the AOTF **410** is controlled electronically by an AOTF controller **420.**

—

The Acousto-Optic Tunable Filter (AOTF) Controller

As shown in **Fig. 4,** the first AOTF controller **420** includes a first DDS driver **424** and a first RF synthesizer card **422** inside computer **50.** The first DDS (Direct Digital RF Synthesizer) driver **424** may be a self-contained unit containing an RF (radio frequency) amplifier and its own power supply. The first DDS driver **424** acts as an interface between the first RF synthesizer card **422** and the first AOTF **410.**

The first RF synthesizer card **422** includes a DDS module which synthesizes and sends a first radio frequency control signal **426** via the first DDS driver **424** to the first AOTF **410.** The DDS module may cooperate with computer software inside the computer **50** to synthesize and send a particular first radio frequency control signal **426.**

Similarly, the second AOTF controller **520** includes a second DDS driver **524** and a second RF synthesizer card **522** inside computer **50.** The second DDS (Direct Digital RF Synthesizer) driver **524** may be a self-contained unit containing an RF (radio frequency) amplifier and its own power supply. The second DDS driver **524** acts as an interface between the second RF synthesizer card **522** and the second AOTF **510.**

The second RF synthesizer card **522** includes a DDS module which synthesizes and sends a second radio frequency control signal **526** via the second DDS driver **524** to the second AOTF **510.** The DDS module may cooperate with computer software inside the

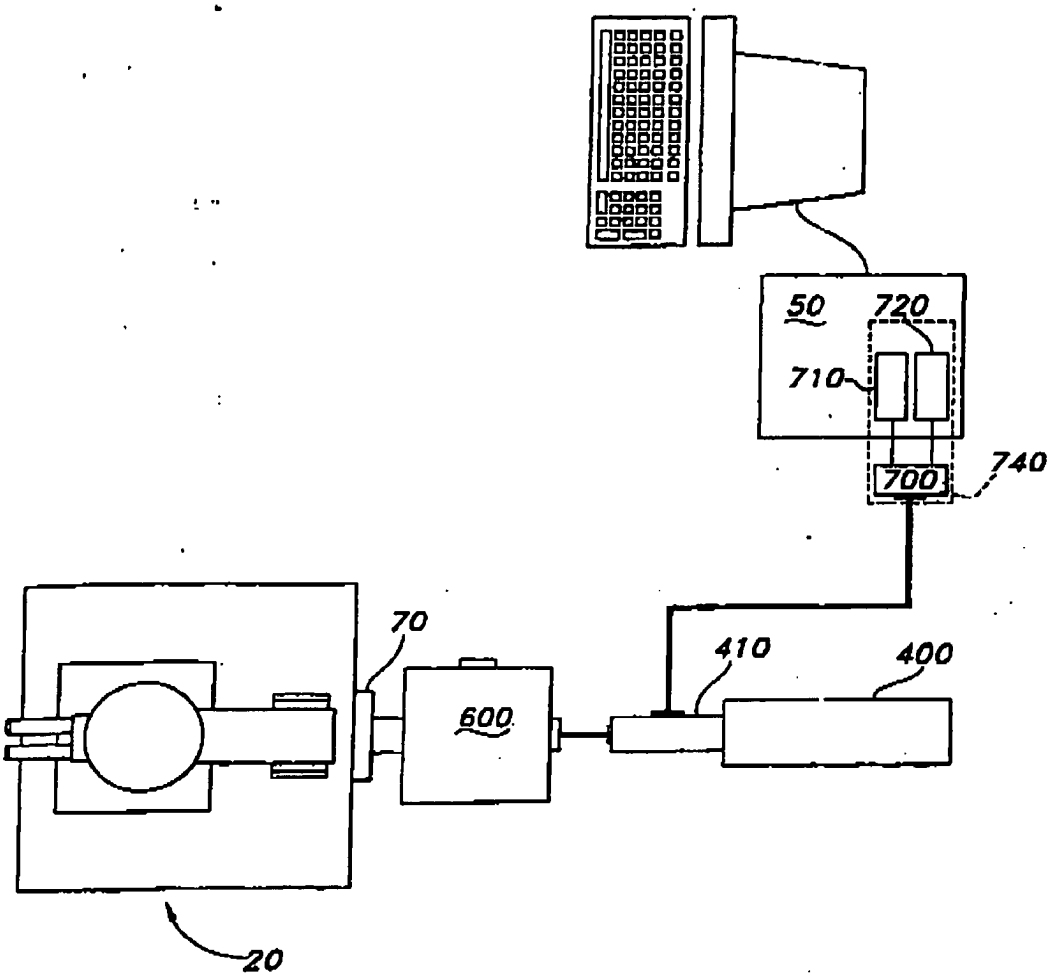

FIG 7

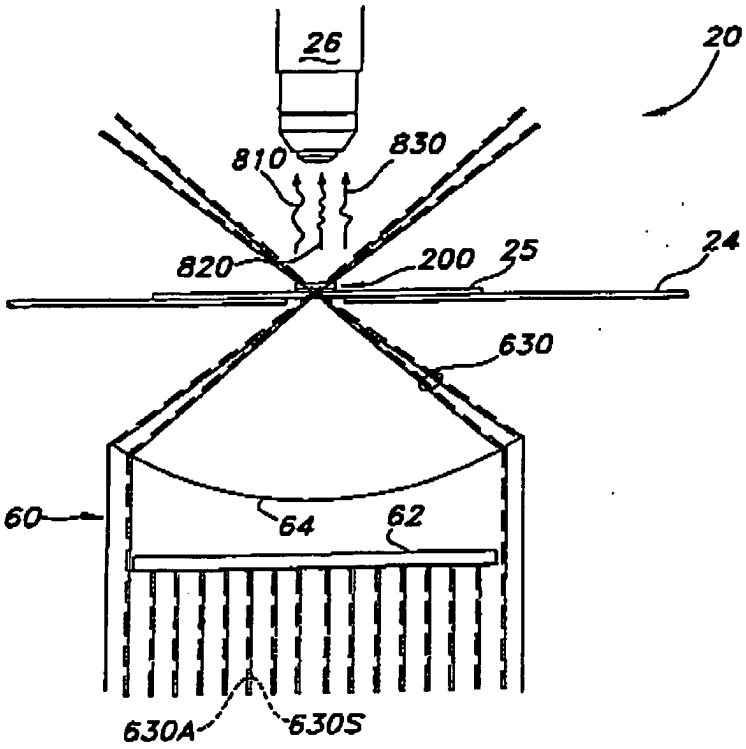

FIG 8

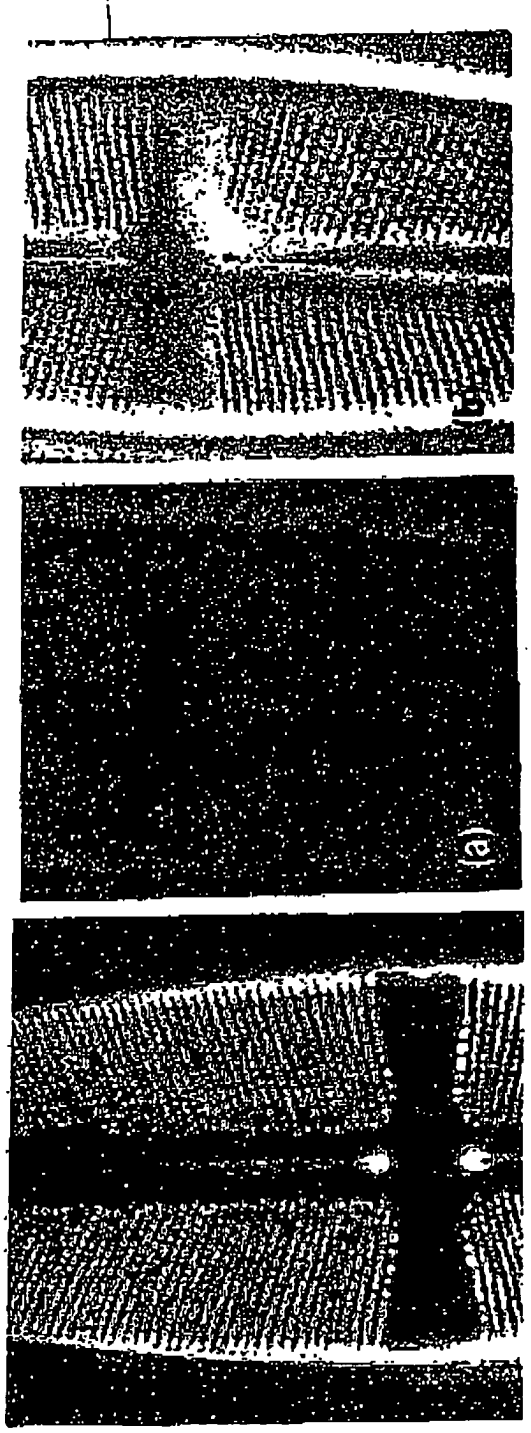

FIG 9B

FIG 9A

FIG 9

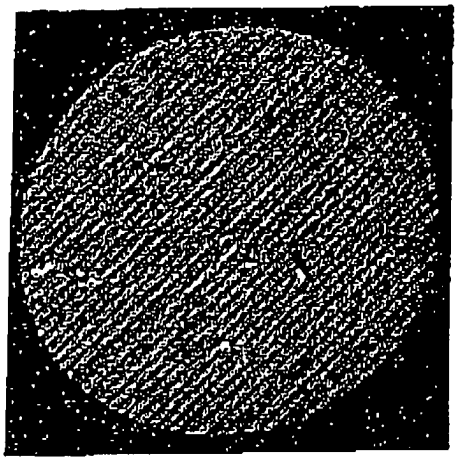

FIG 10C

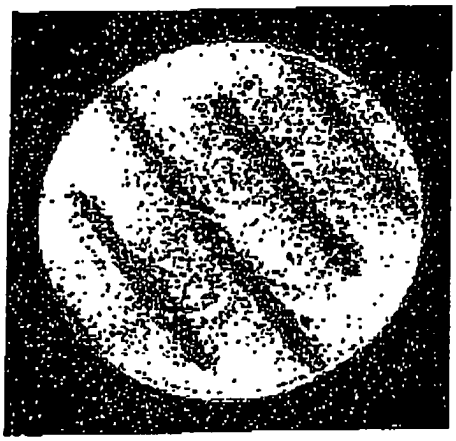

FIG 10B

FIG 10A

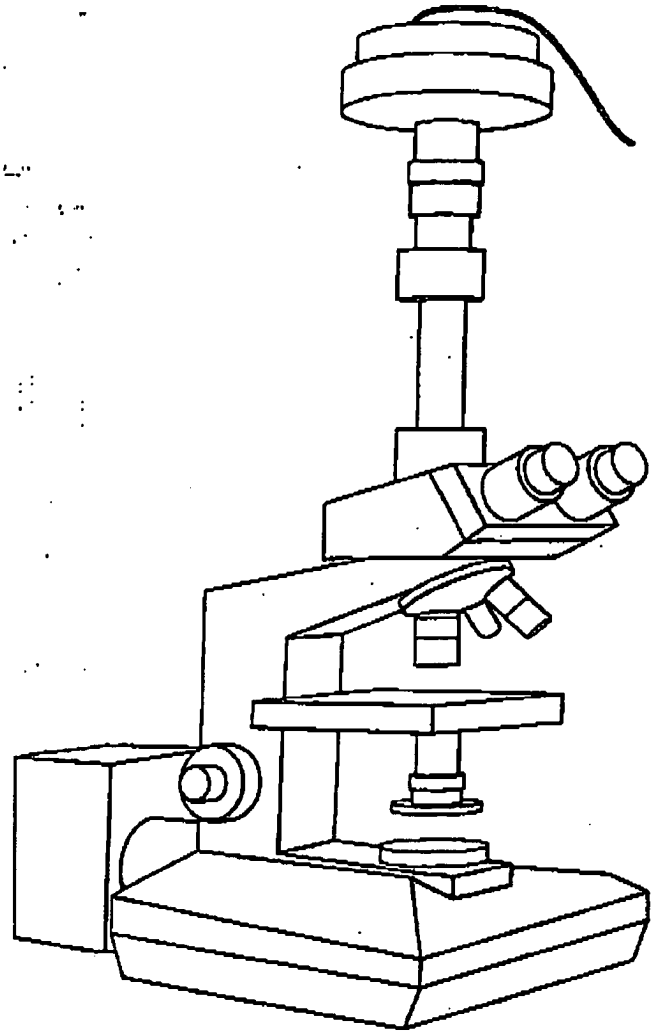

FIG 11

FIG 12

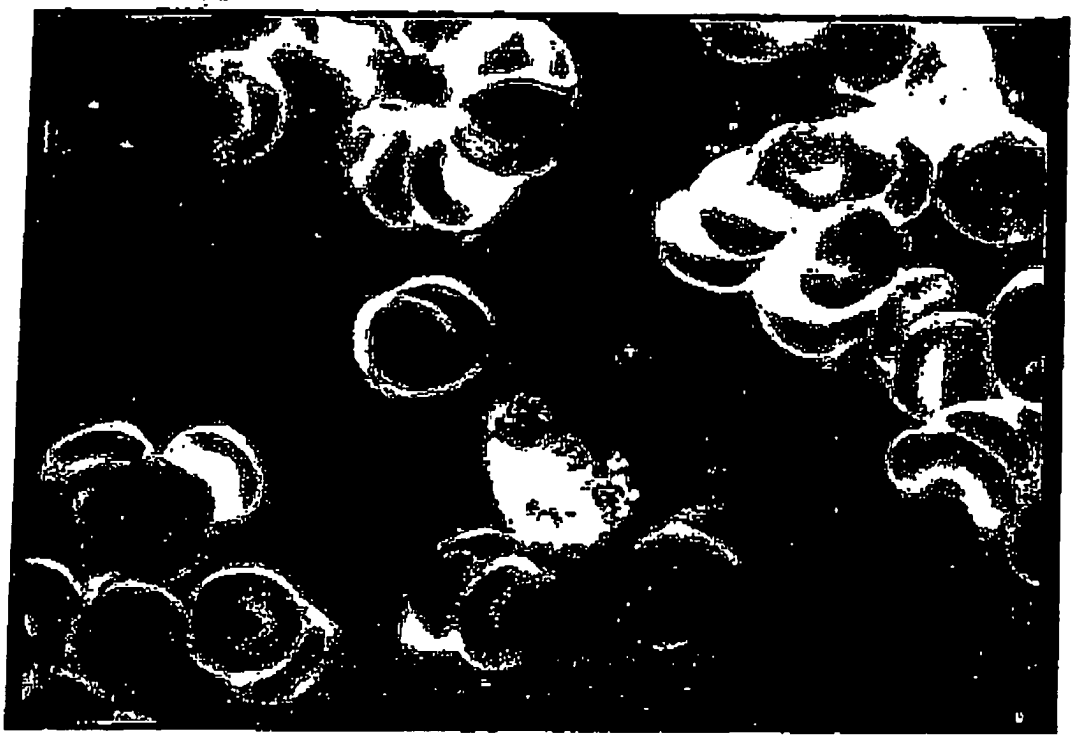

FIG 13

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
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8 August 2002 (08.08.2002)

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(10) International Publication Number
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(26) Publication Language: English

(30) Priority Data:
60/250,800 1 December 2000 (01.12.2000) US

(71) Applicant *(for all designated States except US)*: AUBURN UNIVERSITY [US/US]; 309 Samford Hall, Auburn University, AL 36849-5716 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: VODYANOY, Vitaly, J. [US/US]; 541 Summertrees Drive, Auburn, AL 36832-6766 (US). NEELY, William, Charles [US/US]; 415 Hare Avenue, Auburn, AL 36830-5409 (US).

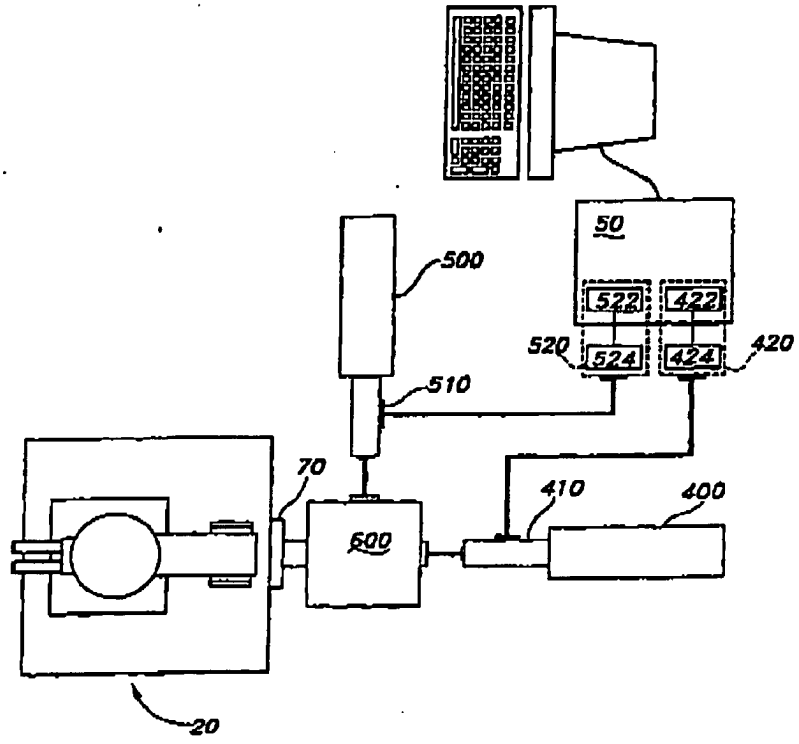
(74) Agents: ANDERSON, J., Scott et al.; Alston & Bird LLP, Bank of America Plaza, Suite 4000, 101 South Tryon Street, Charlotte, NC 28280-4000 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent

[Continued on next page]

(54) Title: HIGH-RESOLUTION OPTICAL MICROSCOPE



(57) Abstract: A direct-view optical microscope system is provided which uses high-energy light from a phenomenon known as non-resonant Raman scattering to illuminate a living biological specimen. One embodiment of the system combines two discrete light sources to form a combined incident light source for the microscope. The system includes a method and apparatus for modulating the intensity of the scattered light when two light waves are combined to produce the incident light. By varying the frequency of the two source light waves, the intensity of the combined Raman-scattered light can be modulated to achieve finer resolution.

WO 02/061485 A3

(BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
27 November 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

Inter I Application No
PCT/US 01/46397

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G02B21/18 G02B21/16 G02B21/33 G02B21/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G02B G01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 45744 A (NORTHERN EDGE ASSOCIATES INC ;RICHARDSON TIMOTHY M (CA)) 15 October 1998 (1998-10-15)	1-4,6-8, 11-13
Y		17-20
A	abstract figures 1-4 page 5, line 19 -page 20, line 10	9,10

-/—

[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 March 2003	02.04.03

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Windecker, R

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Intern I Application No
PCT/US 01/46397

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PATENT ABSTRACTS OF JAPAN vol. 1998, no. 03, 27 February 1998 (1998-02-27) -& JP 09 297266 A (ISHIKAWAJIMA HARIMA HEAVY IND CO LTD;ISHIKAWAJIMA SYST TECHNOL KK), 18 November 1997 (1997-11-18) abstract -& JP 09 297266 A (ISHIKAWAJIMA SYST TECHNOL KK) 18 November 1997 (1997-11-18) figures 1,2	1,3,4, 6-8
Y	US 4 737 022 A (FALTERMEIER BERND ET AL) 12 April 1988 (1988-04-12) abstract column 2, line 65 -column 4, line 15 figure 1	1-8,11, 12
Y	US 5 528 368 A (LEVIN IRA W ET AL) 18 June 1996 (1996-06-18) abstract figures 1,2,6,7 column 1, line 60 -column 2, line 60 column 7, line 3 -column 13, line 34	1-8,11, 12
Y	TOTZECK M ET AL: "PHASE-SHIFTING POLARIZATION INTERFEROMETRY FOR MICROSTRUCTURE LINEWIDTH MEASUREMENT" OPTICS LETTERS, OPTICAL SOCIETY OF AMERICA, WASHINGTON, US, vol. 24, no. 5, 1 March 1999 (1999-03-01), pages 294-296, XP000823521 ISSN: 0146-9592 page 294, left-hand column, last paragraph -right-hand column, paragraph 1 figure 1	1-4,6-8, 11,12
Y	GB 2 195 467 A (AIRE SCIENT LIMITED) 7 April 1988 (1988-04-07) figure 1 page 1, right-hand column, line 123 -page 2, left-hand column, line 4	5
X	US 5 841 577 A (NIU WEN-HUA ET AL) 24 November 1998 (1998-11-24)	23-26
Y	abstract figure 1 column 1, line 19 -column 6, line 29	14-20, 22,31,32

-/—

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Intern Application No
PCT/US 01/46397

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WACHMAN E S ET AL: "IMAGING ACOUSTO-OPTIC TUNABLE FILTER WITH 0.35-MICROMETER SPATIAL RESOLUTION" APPLIED OPTICS, OPTICAL SOCIETY OF AMERICA,WASHINGTON, US, vol. 35, no. 25, 1 September 1996 (1996-09-01), pages 5220-5226, XP000628367 ISSN: 0003-6935	23-26
Y	abstract figure 3A page 5220, right-hand column, paragraph 3 -page 5221, left-hand column, paragraph 1 page 5222, left-hand column, paragraph 4 -page 5223, left-hand column, paragraph 3	14-20, 22,31,32
X	US 4 405 237 A (MANUCCIA THOMAS J ET AL) 20 September 1983 (1983-09-20)	14
Y	abstract figures column 1, line 62 -column 4, line 37	29
X	US 6 108 081 A (ZUMBUSCH ANDREAS ET AL) 22 August 2000 (2000-08-22)	27,28,30
Y		14-20, 22,29, 31,32
A	abstract figure 2 column 1, line 37 -column 8, line 53	21

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 01/46397

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

As a result of the prior review under R. 40.2(e) PCT, no additional fees are to be refunded.

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☒ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1998)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-13

A microscope for observing a specimen comprising a light source, an objective lens, a darkfield condenser, and a compound relay lens having two lenses, wherein the magnification of the first lens of the compound relay lens system is selected to be at least 40 times and the numerical aperture is at least 0.65.

2. Claims: 14-22,31,32

A microscope for observing a specimen comprising an objective lens, a darkfield condenser, and a compound relay lens having two lenses, wherein the illumination is selected to be composed by two light sources and two band-pass filters having different center wavelengths.

3. Claims: 23-26

A system for producing a first and a second light wave having different wavelengths, wherein the two light waves are produced from a single light source.

4. Claims: 27-30

A combiner having a chamber, a casing, and a prisma to combine two light waves, wherein the combiner also comprises a beam expander.

INTERNATIONAL SEARCH REPORT

Inter I Application No
PCT/US 01/46397

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9845744 A	15-10-1998	AU 7019798 A	30-10-1998
		CA 2322800 A1	15-10-1998
		WO 9845744 A2	15-10-1998
		EP 0978008 A2	09-02-2000
JP 09297266 A	18-11-1997	NONE	
US 4737022 A	12-04-1988	DE 3527322 A1	12-02-1987
		JP 62032411 A	12-02-1987
US 5528368 A	18-06-1996	US 5377003 A	27-12-1994
		US RE36529 E	25-01-2000
GB 2195467 A	07-04-1988	NONE	
US 5841577 A	24-11-1998	AU 2128697 A	02-09-1997
		DE 69714021 D1	22-08-2002
		DE 69714021 T2	14-11-2002
		EP 0883829 A1	16-12-1998
		IL 125586 A	23-12-2001
		WO 9730371 A1	21-08-1997
US 4405237 A	20-09-1983	NONE	
US 6108081 A	22-08-2000	CA 2338291 A1	27-01-2000
		EP 1099100 A1	16-05-2001
		JP 2002520612 T	09-07-2002
		WO 0004352 A1	27-01-2000

Form PCT/ISA/210 (patent family annex) (July 1992)

PATENT COOPERATION TREATY

NOV 1 5 2004

PCT Received By

From the INTERNATIONAL SEARCHING AUTHORITY

To:
ALSTON & BIRD LLP
Bank of America Plaza
Attn. Anderson, J. Scott
101 South Tryon Street
Suite 4000
Charlotte, NC 28280-4000
UNITED STATES OF AMERICA

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

Applicant's or agent's file reference: 35721/277803	Date of mailing (day/month/year): 12/11/2004
International application No.: PCT/US2004/017948	FOR FURTHER ACTION See paragraphs 1 and 4 below
Applicant: AUBURN UNIVERSITY	International filing date (day/month/year): 07/06/2004

1. [X] The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

   When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

   Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Fascimile No.: (41-22) 740.14.35

   For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   [ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

   [ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

   The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. These comments would also be made available to the public but not before the expiration of 30 months from the priority date.

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until **30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

   In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

   See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the *PCT Applicant's Guide*, Volume II, National Chapters and the WIPO Internet site.

Name and mailing address of the International Searching Authority: European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer: Cora Dreyer

By
Date 11-15-04

Form PCT/ISA/220 (January 2004) *(See notes on accompanying sheet)*

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 48.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

NOTES TO FORM PCT/ISA/220 (continued)

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international apppfication is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 35721/277803	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US2004/017948	International filing date *(day/month/year)* 07/06/2004	(Earliest) Priority Date *(day/month/year)* 13/06/2003
Applicant AUBURN UNIVERSITY		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
      [ ] The international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
   b. [ ] With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. [ ] **Certain claims were found unsearchable** (See Box II).

3. [ ] **Unity of invention is lacking** (see Box III).

4. With regard to the **title**,
   [X] the text is approved as submitted by the applicant.
   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,
   [X] the text is approved as submitted by the applicant.
   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regards to the **drawings**,
   a. the figure of the **drawings** to be published with the abstract is Figure No. 3
      [X] as suggested by the applicant.
      [ ] as selected by this Authority, because the applicant failed to suggest a figure.
      [ ] as selected by this Authority, because this figure better characterizes the invention.
   b. [ ] none of the figures is to be published with the abstract.

PCT/US2004/017948

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N21/49 G01N21/25 G02B21/18 G01J3/44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N G02B G01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal; WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/061485 A (NEELY WILLIAM CHARLES ; VODYANOY VITALY J (US); UNIV AUBURN (US)) 8 August 2002 (2002-08-08)	1
A	the whole document	3-14
A	US 4 988 630 A (CHEN FANG-CHUNG ET AL) 29 January 1991 (1991-01-29) column 1, line 63 - column 3, line 12	1,2,15, 16
A	US 4 766 083 A (MIYASHITA YOSHINOBU ET AL) 23 August 1988 (1988-08-23) column 2, line 67 - column 3, line 25 column 4, line 7 - line 62	1,2,15, 16
A	US 6 330 058 B1 (LEPARC GERMAN ET AL) 11 December 2001 (2001-12-11) column 3, line 17 - column 4, line 8	1
	-/--	

[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 November 2004	12/11/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Stuebner, B

Form PCT/ISA/210 (second sheet) (January 2004)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4 762 413 A (ANDO OUTARO ET AL) 9 August 1988 (1988-08-09) column 2, line 47 - column 3, line 23	1

Information on patent family members

PCT/US2004/017948

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 02061485 A	08-08-2002	EP 1384103 A2	28-01-2004
		US 2002135871 A1	26-09-2002
		WO 02061485 A2	08-08-2002
		US 2004008522 A1	15-01-2004
		US 2004090669 A1	13-05-2004
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		AT 58245 T	15-11-1990
		DE 3381979 D1	13-12-1990
		EP 0091636 A2	19-10-1983
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		DE 3546566 C2	16-03-1989
		US 4826319 A	02-05-1989

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2004/017948	07.06.2004	13.06.2003

International Patent Classification (IPC) or both national classification and IPC
G01N21/49, G01N21/25, G02B21/18, G01J3/44

Applicant
AUBURN UNIVERSITY

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

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D-80298 Munich
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Authorized Officer

Stuebner, B

Telephone No. +49 89 2399-2179

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2004/017948

Box No. I Basis of the opinion

1. With regard to the language, this opinion has been established on the basis of the international application in the language in which it was field, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2004/017948

Box No. II Priority

1. ☒ The following document has not been furnished:

   ☒ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	2-16
	No:	Claims	1
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-16
Industrial applicability (IA)	Yes:	Claims	1-16
	No:	Claims	

2. Citations and explanations

see separate sheet

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (SEPARATE SHEET)

International application No.
PCT/US2004/017948

Re Item V.

1. The following document is referred to in this communication:
D1 : WO 02/061485 A (NEELY WILLIAM CHARLES ; VODYANOY VITALY J (US); UNIV AUBURN (US)) 8 August 2002 (2002-08-08)

2. INDEPENDENT CLAIM 1

The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claim 1 is not inventive in the sense of Article 33(3) PCT.
Document D1 (see e.g. p.5, l.2 to p.9, l.23) discloses nearly all steps of the method corresponding to Claim 1.
Claim 1 does not explicitly disclose that a "agglutination test" is conducted and: "introducing a quantity of carriers into said sample, each of said carriers configured to attach to said at least one analyte".

However, in D1 (see e.g. p.23, l.33 to p.24, l.34) is disclosed that the known method is used for tests of "living blood cells" and that the method "allows real-time observation with the human eye of biological events taking place at a microscopic, often intracellular level".
Thus, an agglutination test comprising the corresponding well-known steps of measurement is implicitly disclosed in D1.
The subject-matter of Claim 1 therefore lacks novelty or, at least, does not involve an inventive step.

3. DEPENDENT CLAIMS

In Claims 2-16 only slight changes in the method of Claim 1 are defined which come within the scope of the customary practice followed by persons skilled in the art (see also the documents cited in the Search Report), especially as the advantages thus achieved can readily be foreseen.
Consequently, these dependent claims do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements

of the PCT in respect of novelty and/or inventive step.

computer 50 to synthesize and send a particular second radio frequency control signal 526.

The AOTF controllers 420, 520 may be two-channel units such as the acousto-optic tunable filter controller, model VFI-145-70-DDS-A-C2-X, manufactured by Brimrose Corporation of America. It should be understood, however, that any device capable of controlling a device that receives and filters light into a single-frequency light wave may be used as the AOTF controller 420, 520.

The first and second RF control signals 426, 526 are sent by the first and second AOTF controllers 420, 520 to the first and second acousto-optic tunable filters 410, 510. The frequency of the RF control signal 426, 526 determines the frequency of the acoustic wave which is used inside each AOTF 410, 510 to filter the light emitted by each light source 400, 500 into a single-frequency light wave 430, 530.

The Optical T-Combiner

In this embodiment where two light sources 400, 500 are used, the system 10 includes an optical combiner 600 specially designed to combine the lights 430, 530 from two light sources, as shown in Fig. 5. The light sources may be any two of the following: a first light source 400, preferably a laser; a second light source 500, also preferably a laser; and an ultraviolet light source 100. The combiner 600 operates somewhat like a tee connector that might be used in other applications, so it is sometimes referred to as a T-combiner. The combiner 600 preferably includes multiple ports with SMA connectors to receive and transmit the light waves. SMA indicates a Sub-Miniature Type A fiber optic connector.

The combiner 600 of the present invention generally includes a chamber 640 enclosed within a casing 605. A quartz prism 650 inside the chamber 640 combines the two incoming light waves 430, 530. The casing 605 includes three input ports 610, 620, 625 and one output port 615 with SMA connectors. The first and second input ports 610, 620, respectively, are designed to accept input from laser light sources, and a third input port 625 is designed to accept ultraviolet light. With three input ports 610, 620, 625, the combiner 600 is capable of combining any two types of light. Alternative, the combiner 500 will transmit a single light source through the prism 650. The combiner 600 is also capable of transmitting two lights that may

enter through a single port, such as those produced by a dual-channel tunable filter.

Other port configurations and prism types are contemplated and may be used in the combiner **600**, according to the elements present in a particular system, provided the combiner **600** functions to combine two light waves into a single combined light wave **630** capable of provoking the combinatory phenomenon discussed herein.

Each laser input port **610, 620** includes a laser beam expander **612, 622** to focus and collimate (make parallel) the laser beam. A laser beam expander **612, 622** is designed to decrease the laser's beam spot size at large distances. The expander operates like a reverse Galilean telescope, providing a certain angular magnification factor called the expander power. The beam diameter is first increased in size by the expander power. Then, the beam divergence is reduced by the same power. This combination yields a beam that is not only larger, but also one that is highly collimated. The result is an expanded laser beam that produces a smaller beam spot at a large distance when compared to the laser alone. The expanded laser beam also produces smaller beam spot sizes when used in combination with additional focusing optics, a feature that facilitates focusing optimization.

The quartz prism **650** of the optical combiner **600** merges the light waves **430, 530** from two light sources **400, 500**, resulting in a combined light wave **630** that behaves differently from any other single light source. More specifically, the combined light wave **630**, after it passes through the darkfield condenser **60** and strikes the specimen **200**, will produce a combinatory phenomenon.

The Combinatory Phenomenon

The two-source embodiment of the system **10** of the present invention uses the powerful effects of the combinatory phenomenon to improve the resolution **D** of the microscope **20**. When two lights **430, 530** are combined to form a single combined light **630**, the interaction of the two light waves **430, 530** traveling at frequencies f_1, f_2 produces two new combinatory frequencies; namely, a combined additive frequency **Fa** and a combined subtractive frequency **Fs**. As the terms imply, the additive frequency **Fa** equals $f_1 + f_2$ and the subtractive frequency **Fs** equals $f_1 - f_2$. Accordingly, the single

combined light **630** includes two light waves **630A, 630S** traveling at two different frequencies, **Fa** and **Fs**.

The light wave **630A** traveling at the additive frequency **Fa** has greater energy, of course, than the light wave **630S** traveling at the subtractive frequency **Fs**. Accordingly, the additive light wave **630A** will produce the most amount of light scattering and the additive frequency **Fa** will determine the resolution or resolving power **D** of the microscope. The resolution **D** of the microscope **20** in the system **10** of the present invention can be calculated using Abbe's formula (**D** equals **La** divided by twice the **NA**), where **La** is the additive wavelength (corresponding to the additive frequency **Fa**) and **NA** is the numerical aperture of the darkfield condenser **60**.

The resolving power **D** of the microscope **20** in the system **10** of the present invention is an estimate because the intensity of the Raman-scattered light **320** produced by a combined light **630** having an additive wavelength **La** is, to some degree, dependent upon the specimen **200** being viewed.

Example

The interaction of two single-frequency lights **430, 530** may be illustrated by an example. A first light **430** having a first wavelength L_1 of 440×10^{-9} meters is combined with a second light **530** having a second wavelength L_2 of 400×10^{-9} meters. We can calculate the corresponding frequencies f_1, f_2 using Equation One (frequency equals the speed of light divided by the wavelength). The first frequency f_1 equals 6.81×10^{14} Hz. The second frequency f_2 equals 7.49×10^{14} Hz.

Combining light at these two frequencies f_1, f_2 produces a combined light **630** which includes light waves traveling at two different frequencies **Fa, Fs**. Using the frequencies f_1, f_2 calculated, the additive frequency **Fa** ($f_1 + f_2$) equals 14.30×10^{14} Hz and the subtractive frequency **Fs** ($f_1 - f_2$) equals 0.680×10^{14} Hz.

The light waves **630A** traveling at the additive frequency **Fa** of 14.30×10^{14} Hz produce light which is in the ultraviolet range of the electromagnetic spectrum. As shown in **Fig. 6.**, generally, the higher the frequency, the higher the energy. Ultraviolet light has more energy than visible light or light in the very low frequencies such as infrared light, microwaves, and radio waves. The light waves **630S**

traveling at the subtractive frequency **Fs** of 0.680×10^{14} Hz produce infrared light, which has a much lower energy than ultraviolet light.

The resolution **D** of a microscope illuminated by the combined light **630** can be calculated using Abbe's formula (**D** equals **La** divided by twice the **NA**). Using the light waves **630A** traveling at the additive frequency **Fa** of 14.30×10^{14} Hz (and its corresponding additive wavelength **La** of 209×10^{-9} meters) and the numerical aperture **NA** of the darkfield condenser (which, in one embodiment of the system **10** is 1.41), the resolving power **D** of the microscope **20** is 74.1×10^{-9} meters (741 Angstroms).

As shown in **Fig. 8**, the scattering of a light source that has undergone the combinatory phenomenon (such as the combined light wave **630**) includes the scattering of both the additive light wave **630A** and the subtractive light wave **630S**. Accordingly, both light waves **630A, 630S** will produce three types of scattered light: a same-frequency (**Fa, Fs**) Rayleigh component, a high-frequency (**Fa+Δf, Fs+Δf**) component, and a lower-frequency (**Fa–Δf, Fs–Δf**) component. The three scattered light components (**Fs, Fs+Δf, Fs–Δf**) of the subtractive light wave **630S** are not shown in **Fig. 8** because they possess much less energy than the additive light wave **630A**.

The scattering of the additive light wave **630A**, as shown in **Fig. 8**, includes a combined Rayleigh component **810**, a high-frequency combined Raman component **820**, and a low-frequency combined Raman component **830**. The combined Rayleigh-scattered light **810** is emitted at the same frequency (**Fa**) as the additive light wave **630A**. The combined high-frequency Raman-scattered light **820** is emitted at a higher frequency (**Fa+Δf**). The combined lower-frequency Raman-scattered light **830** is emitted at a lower frequency (**Fa–Δf**).

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Modulating Raman-type Scattering of a Combined Light

In the two-light embodiment, the present invention includes a method of modulating or adjusting the intensity of the combined Raman-scattered light **820** when two light waves **430, 530** are combined to produce the combinatory phenomenon. By varying the frequency of the first and second light waves **430, 530**, the intensity of the combined Raman-scattered light **820** can be adjusted to achieve maximum resolving power **D**.

The acousto-optic tunable filters **410, 510** are used to adjust the frequency of the first and second light sources **400, 500**, respectively, to achieve an increase in the intensity of the combined Raman-scattered light **820** emitted by the particular specimen **200** being viewed.

It has been observed that an increase in the intensity of the combined Raman-scattered light **820** results in an increase in resolving power **D**. Also, the use of increased combined light frequency **Fa** necessarily produces a light wave having higher energy. It has also been observed that a high-energy light source produces more of the non-linear and inelastic (Raman) effects of scattered light, which are desirable in the system **10** of the present invention.

It should be noted that the acousto-optic tunable filters **410, 510** may be adjusted to produce a wide variety of light frequencies f_1, f_2, respectively; any combination of which may be optimal for viewing a particular specimen **200**. Different combinations f_1, f_2 will produce different combinatory frequencies **Fa, Fs**, different intensities of combined Raman-scattered light **820** and, therefore, different resolving powers **D** for a particular specimen **200**.

It should also be noted that different combinations of light frequencies f_1, f_2 will produce different relative intensities of combined Rayleigh-scattered light **810** and combined low-energy Raman-scattered light **830**, both of which may alter the effective resolving power **D** of the microscope system **10** for a particular specimen **200**.

In another aspect of the present invention, the first and second light sources **400, 500**, as shown in **Fig. 4**, may be of different types including, without limitation, laser, ultraviolet, x-rays, or visible light. Just as different frequency combinations f_1, f_2 will produce different relative intensities of Raman-scattered light **320**, different types of light sources will produce different results.

In one configuration, the first light source **400** is a laser and the second light source **500** produces ultraviolet light. After being combined in the optical combiner **600**, the combined light **630** enters the microscope **20**. It is theorized that the presence of high-energy harmonics and non-linear waves from the ultraviolet light source will increase the amount and intensity of Raman-scattered light **320**, thereby increasing resolution.

In another configuration, a single laser can be configured using a beam splitter to emit a laser beam into both the first and second acousto-optic tunable filters **410, 510**. Each acousto-optic tunable filter **410, 510** can then filter the laser into two single-wavelength lights **430, 530**.

Two Single-Frequency Light Waves from One Source

In yet another configuration, shown in **Fig. 7**, a single laser source **400** can provide light waves to the acousto-optic tunable filter **410** that is controlled by a dual-frequency AOTF controller **740**.

The dual-frequency AOTF controller **740** includes a dual-frequency DDS driver **700**, a primary RF synthesizer card **710**, and a secondary RF synthesizer card **720**. The dual-frequency DDS (Direct Digital RF Synthesizer) driver **700** may be a self-contained unit containing an RF (radio frequency) amplifier and its own power supply. The dual-frequency DDS driver **700** acts as an interface between the primary and secondary RF synthesizer cards **710, 720** and the AOTF **410**.

The primary RF synthesizer card **710** includes a DDS module which synthesizes and sends a primary radio frequency control signal **716** via the dual-frequency DDS driver **700** to the AOTF **410**. The DDS module may cooperate with computer software inside the computer **50** to synthesize and send a particular primary radio frequency control signal **716**.

Similarly, the secondary RF synthesizer card **720** includes a DDS module which synthesizes and sends a secondary radio frequency control signal **726** via the dual-frequency DDS driver **700** to the AOTF **410**. The DDS module may cooperate with computer software inside the computer **50** to synthesize and send a particular secondary radio frequency control signal **726**.

The dual-frequency driver **700** sends both control signals **716, 726** to the AOTF **410**, which has two channels. The AOTF **410** filters the incoming light from the laser **400** into two single-frequency light waves **430, 530** and broadcasts one on each channel. In use, the dual-frequency driver **700** sends both control signals **716, 726** by alternating; in other words, by repeatedly switching from one frequency to another.

The dual-frequency driver **700**, however, has a maximum switching speed. The excited states of the observed specimen **200**, likewise, have certain lifetimes. Recall that the combined light **630** striking the specimen **200** causes excitation in the molecules of the specimen **200**. The excited states produce the scattered light used to illuminate the specimen **200** in the microscope **20**. If the lifetime of each of the excited states of the specimen **200** is longer than the maximum switching speed, then the dual-frequency driver **700** will operate successfully to produce both light waves **430**, **530**. For a specimen **200** having a very short excitation state, a second AOTF **410** and controller **420** may be needed. Alternatively, a dual-frequency driver **700** with a higher maximum switching speed could be used.

—

Experimental Results

Fig. 9 shows the intricate lattice of a diatom illuminated by an embodiment of the microscope system **10** of the present invention. A diatom is a tiny, unicellular marine organism that has a silica-impregnated outer cell wall sometimes called a lattice. Diatom lattices are often used in microscopy to study and compare systems of illumination and magnification.

The diatom lattice shown in **Fig. 9** was illuminated and photographed using an embodiment of the microscope system **10** of the present invention. The system **10** used to illuminate and photograph the diatom in **Fig. 9** included a 100-watt mercury lamp to produce an ultraviolet light source **100** and included a Naessens darkfield condenser **60** having a numerical aperture **NA** of 1.41 and a 100X objective lens **26**.

Comparing the detail and texture of the diatom lattice in **Fig. 9** to the images in **Figs. 9a** and **9b** illustrates the power of the system **10** of the present invention. **Fig. 9a** is a still photomicrograph taken of a video image of a similar diatom. The image in **Fig. 9b** was enhanced using the gain boost of a Vidicon tube camera.

Figs. 12 and **13** are photomicrographs of living blood cells illuminated by an embodiment of the microscope system of the present invention. Each sample was photographed approximately two minutes after the blood was drawn. Blood cells of different types, red and white, can be seen in motion, interacting with one another.

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Resolution

Micrometers, optical gages, and carbon grating samples are used in microscopy to evaluate, calibrate, and illustrate the resolving power of microscopes. The system **10** of the present invention obtained the images in **Figs. 10a, 10b, and 10c. Fig. 10a** is a photomicrograph of a micrometer with divisions 2.0 microns apart at a magnification of approximately 4,000X. **Fig. 10b** is a photomicrograph of an optical gage with divisions also 2.0 microns apart at a magnification of approximately 7,500X. **Fig. 10c** is a photomicrograph of a carbon grating sample having equidistant and parallel lines of carbon spaced 0.46 microns apart.

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The microscope system **10** of the present invention may find application in numerous fields of scientific study and research including, without limitation, microbiology, bacteriology, virology, general biology, clinical hematology, industrial quality control, reproductive sciences, and any of a variety of other fields where observation of a biological specimen is desired.

The microscope system **10** of the present invention provides a direct-view of the specimen **200**, instead of the indirect views offered by ultraviolet and electron microscopes. The fact that the system **10** includes a direct-view optical microscope **20** allows real-time observation with the human eye of biochemical events taking place at a microscopic, often intracellular level.

The system **10** takes full advantage of the Raman scattering phenomenon as a source of illuminating the specimen **200**, providing a finer resolution and a higher magnification than is currently available from any optical microscope.

The system **10** provides a real-time image of living biological materials, including cells and intracellular structures. Very little specimen preparation is required, leaving living biological specimens unaltered and without artifacts. The system **10** allows observation of living specimens without destroying or altering their biochemical characteristics, and without killing the specimen.

The system **10** also provides a low-cost, low-expertise alternative to the more expensive and complex ultraviolet and electron microscope systems. The system **10** may also be made portable for field operation.

Although the invention has been described in terms of a preferred embodiment, it will be appreciated by those skilled in the art that additions, substitutions, modifications, and deletions not specifically described may be made without departing from the spirit and scope of the invention.

CLAIMS

What is claimed is:

1. A microscope system for observing a specimen, comprising:

an optical microscope having at least one objective lens and at least one eyepiece;

a light source emitting an incident light;

a darkfield condenser positioned to receive said incident light and focus said incident light upon said specimen; and

a compound relay lens connected to said eyepiece.

2. The system of claim 1, wherein said incident light travels at a frequency in the ultraviolet range of the electromagnetic spectrum.

3. The system of claim 1, further comprising:

an adapter positioned between said light source and said microscope, said adapter configured to align said incident light.

4. The system of claim 1, further comprising:

a camera connected to said compound relay lens; and

a computer in communication with said camera,

5. The system of claim 1, wherein said eyepiece comprises an ocular eyepiece pair and a projection eyepiece, and wherein said compound relay lens is connected to said projection eyepiece.

6. The system of claim 1, wherein said compound relay lens comprises:

a first relay lens connected to said eyepiece; and

a second relay lens connected to said first relay lens, said compound relay lens providing higher magnification than a single relay lens alone.

7. A compound relay lens for an optical microscope having at least one eyepiece, comprising:

a first relay lens connected to said eyepiece; and

a second relay lens connected to said first relay lens, said compound relay lens providing higher magnification than a single relay lens alone.

8. The compound relay lens of claim 7, wherein said microscope is connected to a photomicrography system having at least one camera, wherein said second relay lens is positioned between said first relay lens and said camera.

9. The compound relay lens of claim 7, wherein said first relay lens has a numerical aperture of at least 0.65 and a magnification power of at least 40 times.

10. The compound relay lens of claim 7, wherein said second relay lens has a magnification power of at least 10 times.

11. A method of provoking light scattering sufficient to illuminate a specimen in an optical microscope system, said system comprising a visible-light microscope having a darkfield condenser, at least one objective lens, and a compound relay lens, said method comprising:

illuminating a lamp that emits a first light, wherein said first light travels at a frequency in the ultraviolet range of the electromagnetic spectrum;

focusing said first light upon said specimen using said darkfield condenser; and

magnifying the image of said specimen using said compound relay lens.

12. The method of claim 11, further comprising:

adapting said ultraviolet light for use in said microscope by positioning an adapter between said lamp and said darkfield condenser.

13. The method of claim 11, wherein said specimen is placed upon a slide and is covered by a cover glass, said method further comprising:

placing a lower oil drop on the underside center of said slide;

positioning said slide on the center of said darkfield condenser;

placing an upper oil drop on the top center of said cover glass;

raising said darkfield condenser until said upper oil drop contacts said objective lens.

14. A microscope system for illuminating and observing a specimen with scattered light from a combined light source, said system comprising:

an optical microscope having at least one objective lens and at least one eyepiece;

a first light wave traveling at a first frequency;

a second light wave traveling at a second frequency;

an optical combiner positioned to receive said first and second light waves and combine said lights into a combined light, said combined light comprising an additive light wave traveling at an additive frequency and a subtractive light wave traveling at a subtractive frequency;

a darkfield condenser positioned to receive said combined light and focus said combined light upon said specimen such that said additive and subtractive light waves provoke scattered light.

15. The system of claim 14, wherein said first light wave traveling at a first frequency is produced by a first filter system, comprising:

a first light source emitting a first unrefined light wave;

a first filter connected to said first light source and configured to receive said first unrefined light wave;

a first filter controller connected to said first filter, said first filter controller configured to send a first control signal capable of adjusting said first filter such that said first filter refines said first unrefined light wave into said first light wave traveling at said first frequency.

16. The system of claim 14, wherein said second light wave traveling at a second frequency is produced by a second filter system, comprising:

a second light source emitting a second unrefined light wave;

a second filter connected to said second light source and configured to receive said second unrefined light wave;

a second filter controller connected to said second filter, said second filter controller configured to send a second control signal capable of adjusting said second filter such that said second filter refines said second unrefined light wave into said second light wave traveling at said second frequency.

17. The system of claim 14, further comprising a compound relay lens connected to said eyepiece.

18. The system of claim 17, further comprising:

a camera connected to said compound relay lens; and

a computer in communication with said camera,

19. The system of claim 17, wherein said eyepiece comprises an ocular eyepiece pair and a projection eyepiece, and wherein said compound relay lens is connected to said projection eyepiece.

20. The system of claim 17, wherein said compound relay lens comprises:

a first relay lens connected to said eyepiece; and

a second relay lens connected to said first relay lens, said compound relay lens providing higher magnification than a single relay lens alone.

21. The system of claim 20, wherein said first relay lens has a numerical aperture of at least 0.65 and a magnification power of at least 40 times, and wherein said second relay lens has a magnification power of at least 10 times.

22. The system of claim 14, wherein said optical combiner comprises:

a chamber;

a casing enclosing said chamber, said casing comprising a plurality of input ports and an output port; and

a prism assembly positioned within said chamber, said prism assembly configured to receive said light waves entering through any two of said plurality of input ports, to combine said light waves into said combined light wave, and to project said combined light wave through said output port.

23. A system for producing a first light wave traveling at a first frequency and a second light wave traveling at a second frequency from a single light source emitting an unrefined light wave, said system comprising:

a dual-channel filter configured to receive said unrefined light wave;

a dual-frequency filter controller connected to said dual-channel filter and configured to send a primary and a secondary control signal to said dual-channel filter,

said dual-channel filter configured to broadcast said first light wave on a first channel in response to said primary control signal and, in an alternating fashion, to broadcast said second light wave on a second channel in response to said secondary control signal.

24. The system of claim 23, wherein said primary control signal produces a first acoustic wave within said dual-channel filter, said first acoustic wave interacting with said unrefined light wave to produce said first light wave at said first frequency.

25. The system of claim 23, wherein said secondary control signal produces a second acoustic wave within said dual-channel filter, said second acoustic wave interacting with said unrefined light wave to produce said second light wave at said second frequency.

26. The system of claim 23, wherein said dual-frequency filter controller comprises:

a primary radio frequency synthesizer;

a secondary radio frequency synthesizer; and

a driver connecting both of said primary and secondary radio frequency synthesizers to said dual-channel filter,

said primary radio frequency synthesizer configured to synthesize and send a primary control signal via said driver to said dual-channel filter,

said secondary radio frequency synthesizer configured to synthesize and send a secondary control signal via said driver to said dual-channel filter.

27. An optical combiner for combining two light waves to produce a single combined light wave, said optical combiner comprising:

a chamber;

a casing enclosing said chamber, said casing comprising a plurality of input ports and an output port; and

a prism assembly positioned within said chamber, said prism assembly configured to receive said light waves entering through any two of said plurality of input ports, to combine said light waves into said combined light wave, and to project said combined light wave through said output port.

28. The optical combiner of claim 28, further comprising:

a beam expander connected to a first input port designated for light waves emitted by a laser, said beam expander configured to focus and collimate said light waves, said beam expander positioned between said first input port and said prism.

29. The optical combiner of claim 28, wherein a laser beam is received through a first input port and an ultraviolet light wave is received through a second input port, said combiner further comprising:

a beam expander positioned between said first input port and said prism, said beam expander configured to focus and collimate said laser beam.

30. The optical combiner of claim 28, wherein said prism assembly is further configured to receive a single light wave entering through any one of said plurality of input ports, and project said single light wave through said output port.

31. A method of modulating the combinatory phenomenon to illuminate and view a specimen in an optical microscope system with a combined light, said method comprising:

filtering a first unrefined light wave to produce a first light wave traveling at a first frequency;

filtering a second unrefined light wave to produce a second light wave traveling at a second frequency;

combining said first and second light waves to produce said combined light wave, said combined light wave comprising an additive light wave traveling at an additive frequency and a subtractive light wave traveling at a subtractive frequency;

condensing said combined light wave into the shape of a hollow cone;

focusing the first vertex of said hollow cone of combined light upon said specimen.

32. The method of claim 31, wherein said specimen is placed upon a slide and is covered by a cover glass, said method further comprising:

placing a lower oil drop on the underside center of said slide;

positioning said slide on the center of said darkfield condenser;

placing an upper oil drop on the top center of said cover glass;

raising said darkfield condenser until said upper oil drop contacts said objective lens.

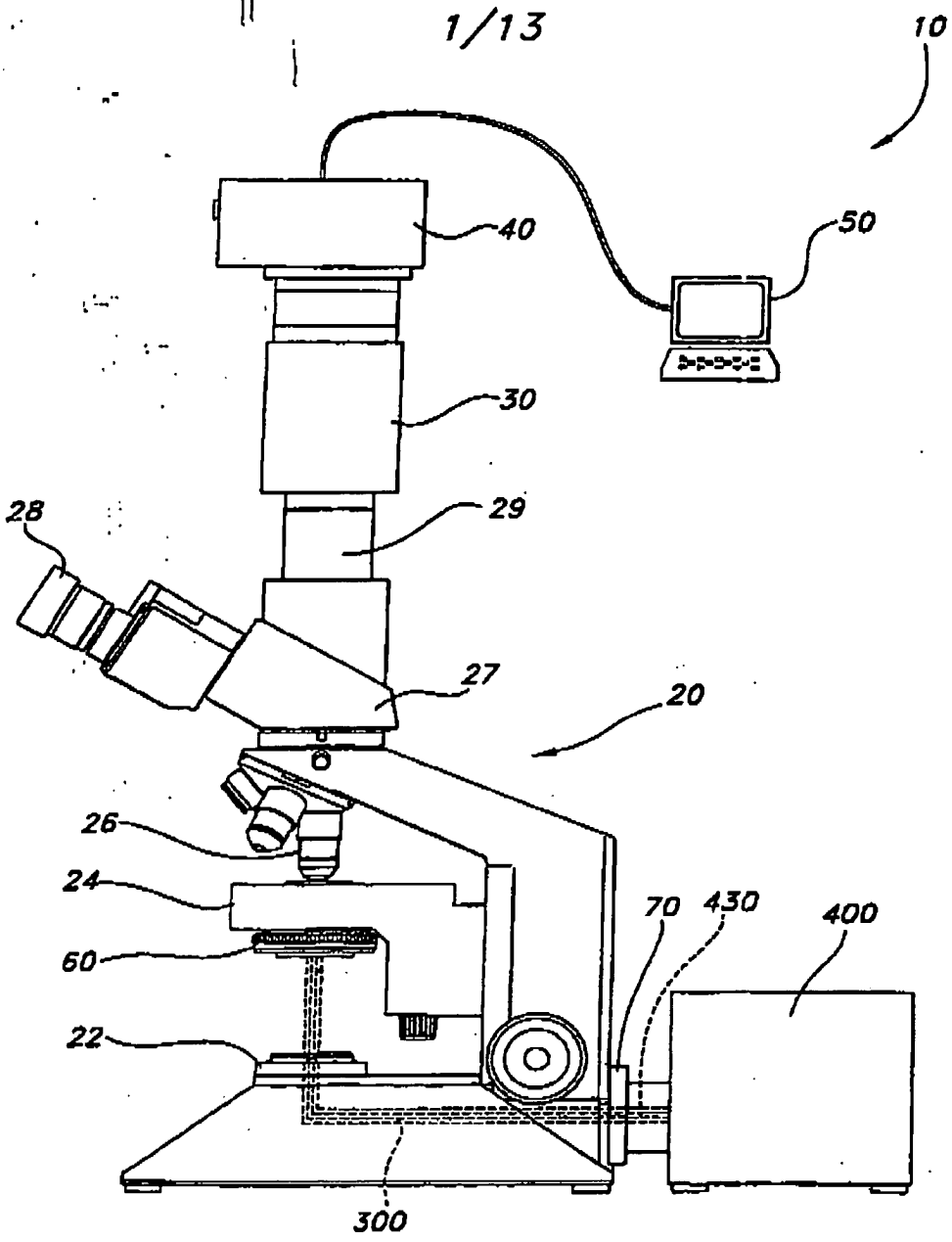

FIG 1

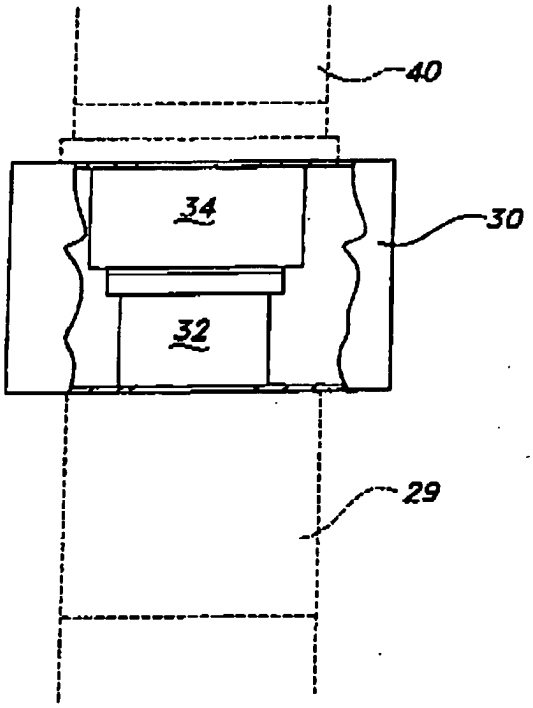

FIG 2

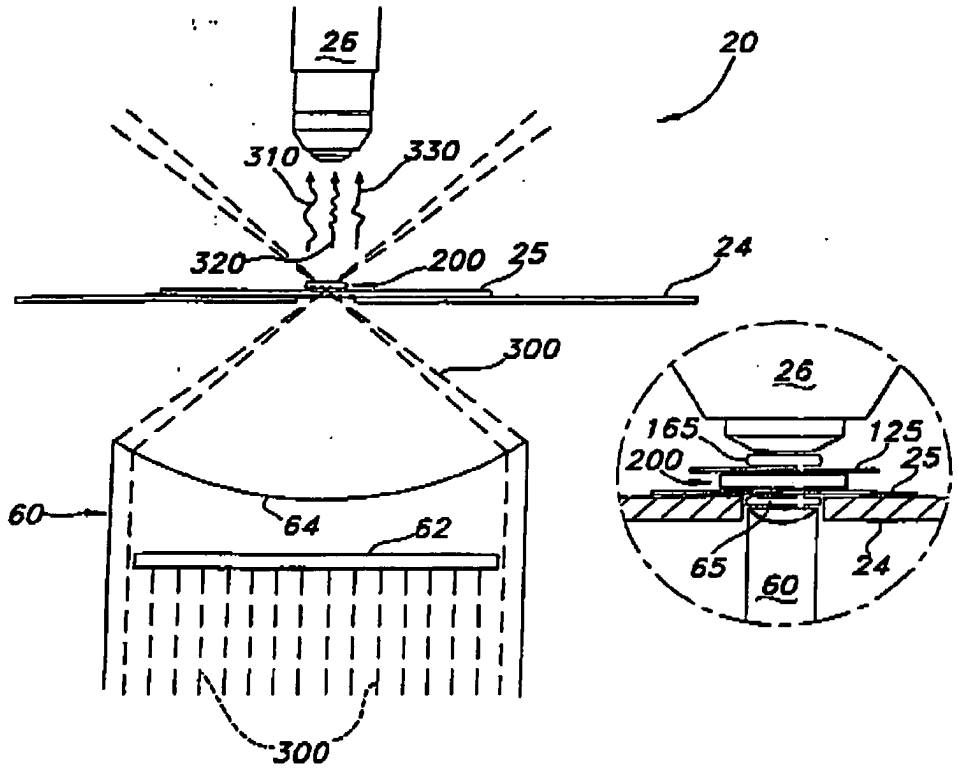

FIG 3

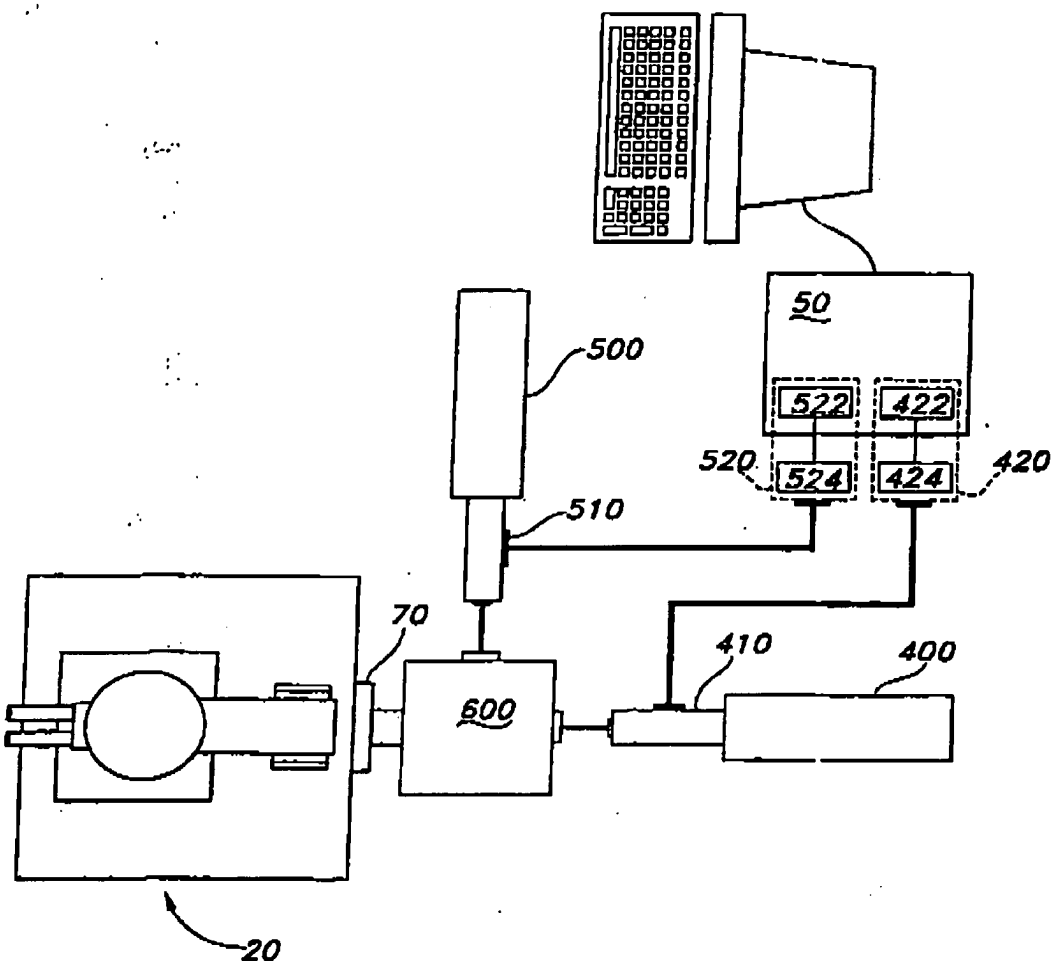

FIG 4

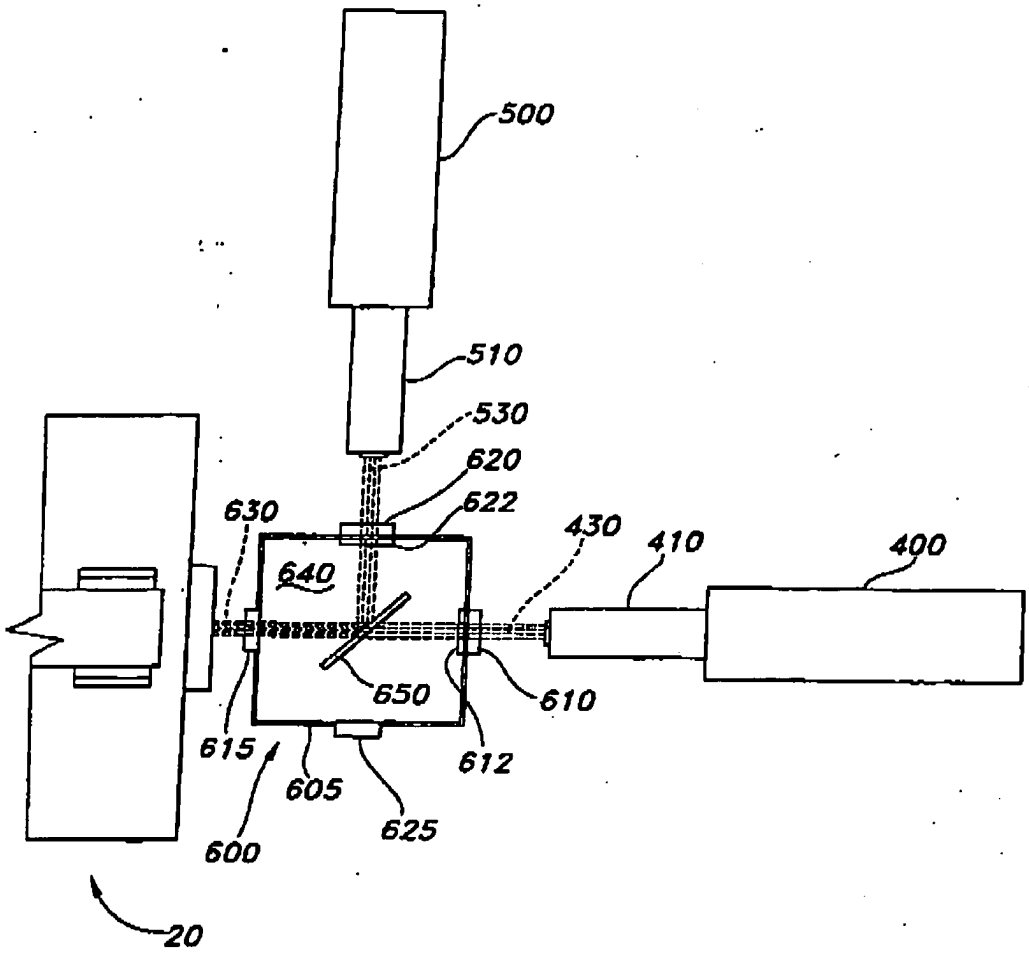
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FIG 5

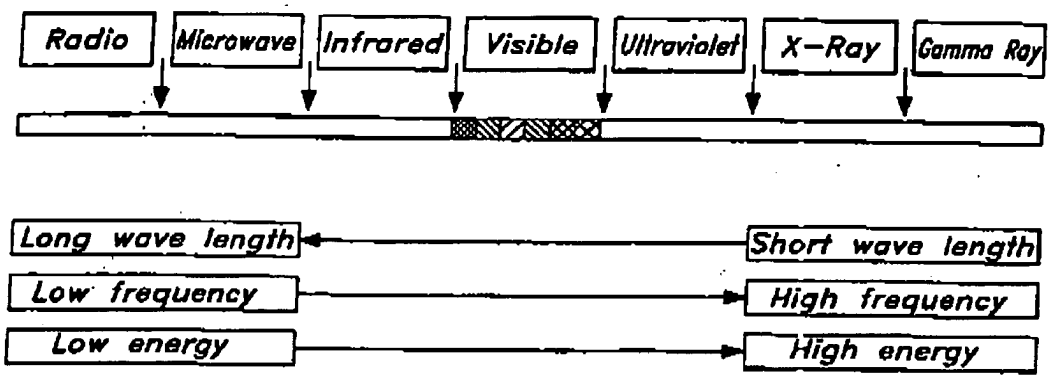

FIG 6